National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 966

Toxic Metals in Bovine Blood

Standard Reference Material (SRM) 966 is intended for use in evaluating the accuracy of lead, cadmium, and total mercury concentration determinations in whole blood. It can also be used for validating analytical methods and for providing traceability to working or secondary blood reference materials containing these constituents. SRM 966 contains frozen whole bovine blood with the aforementioned components at two concentration levels; a base level and an elevated level that contains spiked cadmium, mercury, and methylmercury. A unit consists of two vials of each level; a vial contains approximately 2 mL of whole blood.

The certified, reference, and information values assigned to the base level (Level 1) and the elevated level (Level 2) blood materials are listed in Tables 1 and 2, respectively.

Certified Concentration Values and Uncertainties: Lead concentrations in Level 1 and Level 2 and the cadmium concentrations and total mercury concentrations in Level 2 have been certified. A certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been accounted for or investigated. The certified values are based on measurements using two or more independent analytical methods or a single NIST primary method. Analytical methods used for the characterization of this SRM are given in Table 3. All values are reported in concentration units of μg/L except for lead, which has units of μg/dL. The expanded uncertainties are 95 % confidence intervals and reflect the combined effects of measurement uncertainty, blanks, and any systematic differences between techniques when more than one method has been used [1].

The certified values for lead were determined by a primary method, isotope dilution inductively coupled plasma mass spectrometry (ID-ICPMS). The certified values for cadmium and total mercury concentrations were determined by averaging data from two independent analytical methods. Confirmatory measurements were made by graphite furnace atomic absorption spectrometry (GFAAS) and flow injection atomic absorption spectrometry (FIAAS) at the Centers for Disease Control and Prevention (CDC), Atlanta, GA.

Expiration of Certification: The certification of this SRM is valid until **31 December 2005**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. The certification is nullified if the SRM is damaged, contaminated, or modified.

Maintenance of Certification: NIST will periodically monitor the stability of the SRM and if changes occur that affect the certification before its expiration, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

The overall direction and coordination of the technical measurements leading to the certification of this SRM were carried out by R.D. Vocke, Jr. of the NIST Analytical Chemistry Division.

Analyses were performed by S.J. Christopher, S.E. Long, E.A. Mackey, and M.S. Rearick of the NIST Analytical Chemistry Division and M. Chaudhary-Webb, H.P. Chen, and D.C. Paschal of the CDC, Atlanta, GA.

The experimental design and statistical analysis were provided by W.S. Liggett and M.G. Vangel of the NIST Statistical Engineering Division.

Willie E. May, Chief
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Certificate Issue Date: 21 January 2004
See Certificate Revision History on Last Page

John Rumble, Jr., Chief
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The support aspects involved in the preparation, certification, and issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by J.C. Colbert and P. Fagan of the NIST Meaurement Services Division.

Reference Concentration Value and Uncertainty: A reference value for the concentration of inorganic mercury in Level 2 has been derived from a method-specific protocol [2]. Reference values are noncertified values that represent a best estimate of the true value; however, all known or suspected sources of bias have not been fully investigated at NIST. The uncertainty for the reference concentration of inorganic mercury is the 95 % confidence limit [2] and reflects only the combined effects of measurement uncertainty and variability in concentrations between vials.

Information Concentration Values: Information values for the concentrations of cadmium and total mercury in Level 1 and methylmercury in Level 2 are provided. The methylmercury concentration was not measured and is assumed to be the difference between the total mercury and the inorganic mercury concentrations in Level 2. Information values are provided when insufficient information is available to adequately assess the uncertainty or only a limited number of analyses were performed.

NOTICE AND WARNING TO USERS

Warning: This material is intended for "in vitro" diagnostic use only. It was derived from whole bovine blood collected at a USDA licensed establishment. The supplier of this material has reported that all donor animals were sourced in the United States.

Storage: This SRM should be kept in its original vials and stored frozen at or below -20 °C. The vials should be stored in their original box and aluminized bag. Frost-free freezers should not be employed for storage because of temperature fluctuations.

INSTRUCTIONS FOR USE

The frozen blood in the vial should be allowed to thaw at room temperature (22 °C) before use. The vial should then be mixed by gently rolling, **NOT** shaking, to remix any water that might have separated on freezing. Shaking will cause unwanted bubbles to form at the top of the sample. **DO NOT** use if the blood is clotted. The contents of a vial may be refrozen after having been thawed and a sample withdrawn. Due to possible evaporative losses, it is advisable to discard this SRM if less than one-third of the original blood volume remains (< 0.6 mL).

SOURCE, PREPARATION, AND ANALYSIS

Source of Material: This SRM was prepared in collaboration with the Division of Environmental Health Laboratory Sciences, National Center for Environmental Health of the CDC under the direction of E.J. Sampson, D.T. Miller, and D.C. Paschal. The bovine blood, obtained from a University of Wisconsin facility, came from animals that were bled after oral dosing with gelatin capsules containing lead nitrate.

Preparation of Material: At the CDC, the collected blood was analyzed for lead by GFAAS [3] and pooled to give the base and elevated levels of endogenous lead. The pools were then treated with tripotassium EDTA at a concentration of 1.5 mg/mL as anticoagulant. The Level 2 pool was also spiked with methylmercury iodide, inorganic mercury, and inorganic cadmium. The two levels were then dispensed into 3 mL polyethylene vials. Homogeneity was assessed by analysis of every 200th vial in sequence at each level using standard CDC analytical methods.

Analytical Methods: At NIST, up to 10 randomly selected vials of each level were analyzed by each method together with controls. The entire content of a vial was weighed and analyzed. The results on a per mass basis were converted to a per volume basis using the experimentally determined density of the material: Level 1 - 1.0504 g/mL; Level 2 - 1.0544 g/mL. The element specific analytical methods used in the value assignment of constituents in this SRM are listed in Table 3.

Table 1. Concentration Values for SRM 966 Toxic Metals in Bovine Blood, Level 1[a]

Element	Certified Value	Information Value
Lead	1.56 µg/dL ± 0.05 µg/dL	
Cadmium		< 0.4 µg/L
Mercury (Total)		< 0.06 µg/L

[a] The uncertainty in the certified value for lead is expressed as an expanded uncertainty, U, at the 95% level of confidence, and is calculated according to the method described in the ISO Guide [1]. The expanded uncertainty is calculated as $U = ku_c$ where u_c is intended to represent, at the level of one standard deviation, the effect of within-technique components of uncertainty. The coverage factor, k, for lead is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % level of confidence is 2.36. Information values have no assigned uncertainty.

Table 2. Concentration Values for SRM 966 Toxic Metals in Bovine Blood, Level 2[a]

Element	Certified Values	Reference Value	Information Value
Lead	25.27 µg/dL ± 0.22 µg/dL		
Cadmium	5.22 µg/L ± 0.16 µg/L		
Mercury (Total)	31.4 µg/L ± 1.7 µg/L		
Mercury (Inorganic)[b]		14.1 µg/L ± 1.1 µg/L	
Mercury (Methlymercury)[c]			17.3 µg/L

[a] The uncertainties in the certified values are expressed as expanded uncertainties, U, at the 95 % level of confidence, and are calculated according to the method described in the ISO Guide [1]. The expanded uncertainty is calculated as, $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-technique and within-technique components of uncertainty when two or more independent techniques were used for value assignment. The coverage factor, k, for each analyte is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % level of confidence and is 2.20, 2.00, and 2.78 for lead, cadmium, and mercury (total), respectively.

[b] The concentration and associated uncertainty (95 % confidence limit) for the reference value for inorganic mercury are taken from the data reported in reference [2].

[c] The methylmercury concentration was not measured and has no assigned uncertainty. The concentration is assumed to be the difference between the total mercury and inorganic mercury concentrations.

Table 3. Methods Used for the Analysis of SRM 966[a]

Cadmium	**Isotope dilution inductively coupled plasma mass spectrometry (ID-ICPMS)** **Radiochemical neutron activation analysis (RNAA)** Graphite furnace atomic absorption spectrometry (GFAAS)
Lead	**Isotope dilution inductively coupled plasma mass spectrometry (ID-ICPMS)** Graphite furnace atomic absorption spectrometry (GFAAS)
Total Mercury	**Isotope dilution inductively coupled plasma mass spectrometry (ID-ICPMS)** **Cold vapor atomic absorption spectrometry (CVAAS)** Flow injection atomic absorption spectrometry (FIAAS)
Inorganic Mercury	Flow injection atomic absorption spectrometry (FIAAS)

[a] Methods used for value assignment are shown in a bold faced type; methods used to assign reference or information values, or to corroborate the certified values, are shown in normal faced type.

REFERENCES

[1] *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st Ed. ISO, Geneva, Switzerland (1993); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297, U.S. Government Printing Office: Washington, DC (1994); available at http://physics.nist.gov/Pubs/.

[2] Chen, H.P.; Paschal, D.C.; Miller, D.T.; Morrow, J.C.; *Determination of Total and Inorganic Mercury in Whole Blood by Online Digestion with Flow Injection*; Atomic Spectroscopy, Vol. 19, pp. 176-179 (1998).

[3] Miller, D.T.; Paschal, D.C.; Gunter, E.W.; Stroud, P.E.; D'Angelo, J.; *Determination of Lead in Blood Using Electrothermal Atomization Atomic Absorption Spectrometry with a L'vov Platform and Matrix Modifier*; Analyst, Vol. 112, pp. 1701-1704 (1987).

Certificate Revision History: 21 January 2004 (Editorial changes); 22 December 2000 (Original certificate date).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.